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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C12N 15/00, 1/20, A61K 38/20, C07K 14/52, 16/24 — A1

(11) International Publication Number: WO 95/27052

(43) International Publication Date: 12 October 1995 (12.10.95)

(21) International Application Number: PCT/US95/04094

(22) International Filing Date: 30 March 1995 (30.03.95)

(30) Priority Data:

08/219,831	30 March 1994 (30.03.94)	US
08/224,010	6 April 1994 (06.04.94)	US

(71) Applicant: UNIVERSITY OF MARYLAND AT BALTIMORE [US/US]; 511 West Lombard Street, Baltimore, MD 21202-1691 (US).

(72) Inventors: ALMS, William; 2198 Mt. Hebron Court, Ellicott City, MD 21042 (US). WHITE, Barbara; 9316 Rock Meadow Drive, Ellicott City, MD 21042 (US).

(74) Agents: HUNTINGTON, R., Danny et al.; Burns, Doane, Swecker & Mathis, Washington and Prince Streets, P.O. Box 1404, Alexandria, VA 22313-1404 (US).

(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT, UA, UG, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD, SZ, UG).

Published
With international search report.

(54) Title: HUMAN INTERLEUKIN VARIANTS GENERATED BY ALTERNATIVE SPLICING

(57) Abstract

Novel splice mutants of interleukins-2 and 4 are disclosed, which contain exons 1, 3 and 4 of the full-length mRNAs, but have exon 2 deleted. The proteins resulting from the expression of these splice mutants are useful in regulating the activity of the full-length interleukins.

HUMAN INTERLEUKIN VARIANTS GENERATED BY ALTERNATIVE SPLICING

BACKGROUND OF THE INVENTION

Field of the Invention

This invention relates to novel splice mutants of interleukins, which contain deletions of one or more exons, the expression of which results in truncated proteins which are useful in regulating the action of their full-length counterparts.

Description of the Related Art

Interleukin-4 is a 15 kDa glycoprotein secreted by activated T cells, (Howard et al. (1982) *J. Exp. Med.* 155:914), mast cells (Brown et al. (1987) *Cell* 50:809) and basophils (Seder et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:2835) which regulates a wide spectrum of cellular functions in hematopoietic and nonhematopoetic cells. The sequence of IL-4 is disclosed in U.S. Patent No. 5,017,691.

Recently the 3 dimensional structure of IL-4 has been solved (Powers et al. (1992) *Science* 256:1673). The protein contains 4 left hand α-helices and two ß sheets. This structural motif is shared by a growing group of

of left-handed alpha-helices and B sheets similar to that of IL-4 (Bazan (1992) *Science* 257:410). Exon 2 of IL-2 (amino acid residues 31 to 50) encodes a B sheet, a short α helix, and the loop connecting helices α_A and α_B (Bazan (1992) *Science* 257:410), a region which is similar to that encoded by exon 2 of IL-4 (Powers et al. (1992) *Science* 256:1673). Exon 2 of IL-2 encodes the portion of the IL-2 molecule that binds the α chain (p55) of the IL-2 receptor (Sauve et al. (1991) *Proc. Natl. Acad. Sci. USA* 88:4636).

IL-4 has been shown to co-stimulate proliferation of resting B cells with anti-IgM antibodies (Howard et al. (1982) *J. Exp. Med.* 155:914), rescue resting B cells from apoptosis (Illera et al. (1993) *J.Immunol.* 151:3521), induce Ig production by activated B cells (Defiance et al. (1988) *J. Immunol.* 141:2000), and regulate isotype switching to IgG_1 and IgE in mice (Coffman et al. (1986) *J. Immunol.* 136:4538) (Vitetta et al. (1985) *J. Exp. Med.* 162:1726), and IgG_4 and IgE in humans (Lundgren et al. (1989) *Eur. J. Immunol.* 13:131). IL-4 exposure has been demonstrated to increase the number of IgM (Shields et al. (1989) *Immunology* 66:224), CD23 (10-12), MHC class II molecules (Rousset et al. (1988) *J. Immunol.* 140:2625) (Roehm et al. (1984) *J. Exp. Med.* 160:679), LFA-1 and LFA-3 (Rousset et al. (1989) *J. Immunol.* 143:1490), and IL-4 receptor (IL-4R) (Renz et al. (1991) *J. Immunol.* 146:3049) molecules on the surface of B cells. In T

(T_C). T_C blast cells express surface receptor for IL-2. The IL-2 receptor (IL-2) is composed of 3 separate proteins p55 (α chain), p75 (β chain), and p65 (δ chain). In different combinations, these chains give rise to various forms of the IL-2R with different affinities and capacity to transduce proliferative signals (Taniguchi et al. (1993) *Cell* 73:5). Similarly, the IL-4R consists of at least two chains. The first IL-4R chain which was described shares significant homology to the β chain of the IL-2R and other members of the growth factor receptor superfamily (Idzerda et al. (1990) *J. Exp. Med.* 171:861). Very recently, a second IL-4R chain was identified, which is the δ_C chain of the IL-2R (Russell et al. (1993) *Science* 262:1877). IL-4R, like IL-2R, may have several functional forms (Rigley et al. (1991) *Int. Immunol.* 3:197).

Because of the widespread effects of IL-4, it is not surprising that the regulation of IL-4 activity is pivotal in determining the outcome of certain diseases (Scott et al. (1988) *J. Exp. Med.* 168:1675) (Heinzel et al. (1989) *J. Exp. Med.* 169:59) (Yamamura et al. (1991) *Science* 254:277) (Zwingenberger et al. (1991) *Scand. J. Immunol.* 34:243) (Wierenga et al. (1990) *J. Immunol.* 144:465). In murine leishmaniasis (Heinzel et al. (1989) *J. Exp. Med.* 169:59), human leprosy (Yamamura et al. (1991) *Science* 254:277), and human schistosomiasis (Zwingenberger et al. (1991) *Scand. J. Immunol.* 34:243),

Yet a further object of the present invention is to provide antibodies to the polypeptides resulting from the expression of the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4.

Another object of the present invention is to provide a method of regulating the activity of human IL-2 and 4 by administering an amount of the polypeptides resulting from the expression of the isolated nucleic acids containing exons 1, 3 and 4 of human IL-2 and 4, respectively, effective to decrease the biological effects of human IL-2 and 4, respectively.

With the foregoing and other objects, advantages and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference to the following detailed description of the preferred embodiments of the invention and to the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the detection of two IL-4 mRNA species. Total cellular RNA was extracted from human peripheral blood mononuclear cells (PBMC) stimulated for 6 hours with the anti-CD3 MAb, OKT3, then subjected to reverse transcriptase-polymerase chain reaction (RT-PCR) using oligonucleotide primers specific for exons 1 and 4 of human IL-2, exons 1 and 4 of human IL-4, and interferon-δ (IFN-γ). IL-2, IL-4, and IFN-γ cRNA

pCR™II vector and their DNA sequences determined using the dideoxy-mediated chain termination method (41). Sequence analysis of IL-4δ2 cDNA demonstrated the presence of IL-4 exons 1, 3 and 4, with exon I spliced directly to exon 3, in frame. Sequence analysis of IL-4 cDNA isolated, cloned, and sequenced in parallel with IL-4δ2 cDNA demonstrated the expected presence of exons 1, 2, 3 and 4. An autoradiogram of the sequencing gel at the region of the IL-4δ2 exon 1-exon 3 splice junction is shown.

Figure 4 shows RNase protection of IL-4 and IL-4δ2 RNA. A radiolabeled IL-4δ2 probe containing an IL-4 exon 1-exon 3 junction was purified and hybridized to 15-20 μg of denatured total cellular RNA from activated PBMC or yeast tRNA. Unhybridized RNA was digested with RNase TI, and the protected RNA fragments were size separated in a 6% denaturing polyacrylamide gel and subjected to autoradiography. Lane 1 shows molecular weight markers, lane 2 shows the purified IL-4δ2 probe, lane 3 shows protection of total cellular RNA from activated PBMC, and lane 4 shows protection of tRNA as a negative control. The 342 bp band in lane 2 represents protected IL-4δ2 RNA and the faint 279 bp band represents protected IL-4 RNA.

Figure 5 shows expression of IL-4 and IL-4δ2 mRNAs in different ratios in different healthy donors. PBMC from 3 healthy individuals were stimulated with anti-CD3 MAb for 6 hours. Expression of IL-4 and IL-4δ2 mRNAs was

4-specific oligonucleotide primers. The 5′ PCR oligonucleotide primer was end-labeled with ^{32}P. The RT-PCR amplification products were subjected to gel electrophoresis in a 6% polyacrylamide gel. An autoradiogram of the gel is shown, in which lane 1 = 0 hours, lane 2 = 3 hours, lane 3 = 6 hours, lane 4 = 8 hours, lane 5 = 12 hours, and lane 6 = negative control RT-PCR products.

Figure 8 shows that mice do not produce IL-4δ2 mRNA. Spleen cells from BALB/c mice were stimulated with PMA and ionomycin for 24 hours. RNA was extracted and subjected to RT-PCR using murine IL-4 exon 1- and exon 4-specific primers. Human IL-4 and IL-4δ2 mRNA expression was assayed in parallel from anti-CD3 MAb stimulated PBMC with human IL-4 exon 1 and exon 4-specific primers. The RT-PCR products were subjected to agarose gel electrophoresis and detected with ethidium bromide staining. IL-4, but not IL-4δ2, mRNA expression was observed in the murine spleen cells (lane 2), whereas human PBMC expressed both IL-4 and IL-4δ2 mRNA (lane 2). Lane M contains molecular weight markers.

Figure 9 shows the detection of two IL-2 mRNA species. Total cellular RNA was extracted from human PBMC stimulated for 6 hours with the anti-CD3 MAb, OKT3, then subjected to RT-PCR using oligonucleotide primers specific for exons 1 and 4 of human IL-2. In panel A, the 5′ PCR oligonucleotide primer was end-labeled with

Figure 11 shows the complete sequence of the IL-2 gene (SEQ ID NO:25) (Fujita et al, *Proc. Natl. Acad. Sci.*, Vol. 80, pp. 7437-7441 (1983)). The IL-2δ2 (SEQ ID NO:26) of the present invention contains the sequences encoded by exons 1, 3 and 4, but not 2.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

The present invention demonstrates the expression of IL-4δ2, a second mRNA isoform transcribed from the IL-4 gene by alternative splicing. Alternative splicing is an efficient mechanism by which multiple protein isoforms may be generated from a single genetic locus. Protein isoforms generated by this regulatory mechanism may vary in function, cellular localization, or pattern of developmental expression (Smith et al. (1989) *Annu. Rev. Genet.* 23:527). Alternative splicing is used in terminally differentiated cells to reversibly modify protein expression without changing the genetic content of the cells (Smith et al. (1989) *Annu. Rev. Genet.* 23:527).

IL-4δ2 was first observed as an additional RT-PCR amplification product during analysis of cytokine gene expression. Cloning and sequencing of the cDNA demonstrated that IL-4δ2 consists of exons 1, 3 and 4 of the IL-4 gene, but not exon 2. Splicing of exon 1 to exon 3 occurs in IL-4δ2 mRNA without changing the reading

specific for CD4, and δTCS1, specific for Vδ1 - Jδ1 and Vδ1 - Jδ2.

Following binding of the MAb to the cells, the cells can be treated with a second antibody specific for the first antibody, which is either coupled to a separation medium, or which can be coupled to a separation medium via a particular linkage, such as a biotin-avidin linkage. Particularly preferable for the present invention is a sheep - anti-mouse IgG coupled to a support such as Dynabeads M-450 (Dynal).

Once the cells are separated, they are cloned in the presence of mitogens, growth factors and/or feeder cells. Preferable mitogens include but are not limited to phytohemagglutinin (PHA) at a concentration of 1-100 μg/ml, preferably at about 10μg/ml. Preferable growth factors include but are not limited to IL-2, at a concentration of 1-100 U/ml, preferably about 50 U/ml. Preferable feeder cells include but are not limited to allogeneic PBMC, preferably irradiated at 1000-10,000 rad, preferably at about 3,000 rad. The cells may also be treated with supernatant from a hybridoma cell line, preferably OKT3, which may stimulate T cell proliferation.

The cells can be grown in any suitable medium, but RPMI is preferable. The medium is preferably supplemented with serum, such as human serum, preferably human male AB serum, and/or fetal calf serum (FCS). The

The amplified isolated DNA can then be ligated into a vector suitable for sequencing, transformed into competent cells, and DNA prepared therefrom. Isolation of such plasmids is by techniques well known in the art. The DNA inserts can then be sequenced using any method known in the art, including Maxam-Gilbert sequencing, or preferably by the dideoxy chain termination reaction of Sanger et al.

The RNA of interest can be identified using any means known in the art, but particularly preferable is an RNA protection assay. According to this method, a radiolabelled probe is made which will bind to the RNA of interest. The radiolabelled probe is incubated with total cellular RNA, and unhybridized RNA is digested using RNase. Upon hybridization of the labelled probe to the RNA of interest, the RNA of interest is protected from the RNase and can be identified by electrophoresis on a polyacrylamide gel, with subsequent autoradiography.

Likewise, the cDNAs prepared can be characterized by Southern blot wherein the DNA of interest is run on an agarose gel, the nucleic acids on the gel are transferred to a nylon or nitrocellulose membrane, and the membrane is hybridized with a probe which will aid in the characterization of the DNA. Particularly preferable for the present invention is a probe which spans the exon/exon junctions of an interleukin. Such probes are then able to identify alternative splice mutants.

demonstrates that the second band is related to IL-4 using an independent method, an RNase protection assay. The present invention also provides sequence data for the entire protein encoding region to definitively show that the molecule is identical to IL-4, except for the omission of exon 2.

The sequence data disclosed herein show that IL-4 exon 2 functions as a cassette exon (Smith et al. (1989) *Annu. Rev. Genet.* 23:527), and that no shift in the reading frame occurs when it is omitted. The RNase protection assay demonstrates that the IL-4δ2 transcript is expressed in the same sense orientation as IL-4 transcripts, because an anti-sense probe was used for protection.

Also determined was whether the alternative splicing of exon 2 was unique to IL-4 mRNA or part of a more general regulatory mechanism for cytokines. The cytokines tested were IL-2, -3, -5, and GM-CSF, which share protein folding motifs, genomic organization, and receptor extracellular binding domains with IL-4 (Boulay et al. (1992) *J. Biol. Chem.* 267:20525). The present invention also demonstrates that IL-2, but not IL-3, IL-5, or GM-CSF, also uses alternative splicing of exon 2. Both IL-2 and IL-4 splice variants omit exon 2, which encode similar regions of secondary structure and participate in receptor binding for each molecule.

the size of IL-4 exon 2. Sequence data of cloned cDNA demonstrates that this variant contains IL-4 exons 1, 3 and 4, with exon 1 spliced directly to exon 3 in an open reading frame. The entire protein encoding region of this variant, named IL-4δ2, is identical to IL-4, except for the omission of exon 2. IL-4δ2 mRNA is detected in all human PBMC and T cell clones tested, but is absent from mouse spleen cells. Amounts of both IL-4 and IL-4δ2 mRNAs increase upon T cell activation, although IL-4 mRNA increases to a greater extent than does IL-4δ2 mRNA. Similar experiments suggest that humans also express a variant of IL-2 mRNA, in which exon 2 is deleted by alternative splicing. Human IL-3, IL-5, and GM-CSF do not use alternative splicing to delete exon 2. Thus, variants of both human IL-4 and IL-2 exist in which similar structural regions of each molecule are omitted by alternative splicing of mRNA.

The following examples are presented in order to more fully illustrate the preferred embodiments of the invention. They should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

Cell Separation and T Cell Cloning.

Human PBMC were isolated from healthy donors by density gradient centrifugation using Histopaque 1077 (Sigma Chemical Co., St Louis, MO). A CD4+ α/β T cell

and PE-conjugated anti-human Leu-4 (CD3) MAb (Becton Dickinson), using standard techniques.

EXAMPLE 2

T Cell Stimulation.

PBMC (5×10^6) or 5×10^6 cloned T cells plus 2.5×10^6 irradiated (3000 rad) allogeneic PBMC were stimulated in 2 ml cultures in complete tissue culture media supplemented to a final concentration of 10% with supernatant of the anti-CD3 MAb secreting hybridoma, OKT3 (American Type Culture Collection, Rockville, MD). This concentration of OKT3 supernatant had previously been determined to optimally stimulate T cell proliferation.

EXAMPLE 3

RNA Isolation and RT-PCR.

Total cellular RNA was isolated from PBMC, T cell clones, and BALB/c spleen cells by acid guanidinium thiocyanate-phenol chloroform extraction (Chomczynski et al. (1987) *Anal. Biochem.* 162:156). One µg of RNA was denatured for 5 minutes at 65°C and then reverse transcribed into cDNA using in a 15 µl reaction mixture containing 200 U of M-MLV reverse transcriptase [Bethesda Research Labs (BRL), Bethesda, MD], 50 mM Tris-HCl, pH 8.3, 75 mM KCl, 8 mM DTT, 3 mM $MgCl_2$, 0.5 mM each dATP, dCTP, dGTP, dTTP (Pharmacia LKB Biotechnology, Piscataway, NY), 1 U/ml RNasin (Promega, Madison, WI),

5′-TCTTCCTGCTAGCATGTGC-3′ [SEQ ID NO: 5] and exon 4 reverse 5′-CGTACTCTGGTTGGCTTTCC-3′ [SEQ ID NO: 6]; human IL-4 pair B exon 1 forward 5′-AAGCTTATGGGTCTCACCTCCCAAC-3′ [SEQ ID NO: 7] and exon 4 reverse 5′-GGATCCTCATCAGCTCGAACACTTTGA-3′ [SEQ ID NO: 8]; murine IL-4 exon 1 forward 5′-AGCCATATCCACGGATGCGAC-3′ [SEQ ID NO: 9] and exon 4 reverse 5′-CTCAGTACTACGAGTAATCCAT- 3′ [SEQ ID NO: 10]; human IL-5 exon 1 forward 5′-CTTTTTGCAAAAGCCTTGGCCTCCAAAAAAGC-3′ [SEQ ID NO: 11] and exon 4 reverse 5′-CCATTCTCCGCCCCAAGGCTGACTAATTTTT-3′ [SEQ ID NO: 12]; human GM-CSF exon 1 forward 5′-ATGTGGCTGCAGAGCCTGCTGCTC-3′ [SEQ ID NO: 13] and exon 4 reverse 5′TCACTCCTGGACTGGCTCCCAGCA-3′ [SEQ ID NO: 14]; and human IFN-γ forward 5′CAGCTCTGCATCGTTTTGGGTTCT-3′ [SEQ ID NO: 15] and reverse 5′-TGCTCTTCGACCTTGAAACAGCAT-3′ [SEQ ID NO: 16]. *BamHI* and *Hind*III restriction enzyme recognition sequences are underlined in the human IL-4 pair B primers. Construction of the IL-2, IL-4 and IFN-γ cRNA internal standards are described in (Alms, W.J. et al. which is hereby incorporated by reference in its entirety).

EXAMPLE 4

Cloning of RT-PCR Products and DNA Sequencing.

Complementary DNAs for IL-4 and IL-4δ2 were generated and amplified by RT-PCR using IL-4 exon 1 and 4 specific primers containing digestion sites for *BamHI* and

EXAMPLE 5

RNase Protection Assays.

A 362 bp IL-4δ2 RT-PCR fragment that spanned IL-4 exon 1 to exon 4 with an exon 1-3 junction was cloned into the pCR™ II vector. The insert orientation was determined by sequence analysis. An RNase protection assay was performed using Ambion RPA II™ (Ambion Inc., Austin, TX), according to the manufacturer's protocol. Briefly, radiolabeled IL-4δ2 probe was generated by incubating 100 ng of *SpeI* linearized IL-4δ2-containing plasmid with 5 units T7 RNA polymerase (BRL), 0.5 mM each ATP, CTP, and GTP, 12 μM UTP and 6 μM 400 Ci/mmol 5'[α-^{32}P]-UTP (Dupont NEN, Boston, MA) for 45 min at 37°C. The final specific activity of the IL-4δ2 probe was 1 x 10^9 cpm/μg DNA. The radiolabeled probe was subjected to gel electrophoresis in a 6% denaturing polyacrylamide gel, and the full length IL-4δ2 probe was identified by autoradiography. The band containing the probe was excised from the gel, and the IL-4δ2 probe was eluted at 37°C in 400 μl buffer containing 2 M ammonium acetate, 1% SDS and 25 μg/ml yeast transfer RNA (tRNA). The radiolabeled IL-4δ2 probe (1 x 10^6 cpm) was hybridized with 15-20 μg of denatured total cellular RNA or tRNA for 16 hours at 37°C in 80% formamide, 40 mM PIPES, pH 6.4, 400 mM NaCl, and 1 mM EDTA buffer. Unhybridized RNA was digested at 30°C for 30 minutes with 200 μl RNase

amplification products were next transferred to nylon membranes by blotting overnight in 20x SSC buffer. The DNA samples were cross-linked to the membrane by UV light irradiation. Membranes were prehybridized in 6x SSC, 10x Denhardt's solution, 0.1% SDS and 50 μg/ml sperm DNA for at least 1 hour at 42°C and then hybridized overnight with 0.2 μg ^{32}P 5′ end-labeled oligonucleotide probe at 49°C in 6x SSC and 1% SDS. The membrane was washed three times in 6x SSC and 1% SDS for 10 minutes at room temperature, followed by a final 49°C wash. Membranes were then subjected to PhosphorImager analysis (Molecular Dynamics, Sunnyvale, CA) or subjected to autoradiography. Cytokine specific oligonucleotide probe sequences were: human IL-2 exon 2-specific 5′-CTCACCAGGATGCTCACA-3′ [SEQ ID NO: 18]; human IL-2 exon 3-specific 5′-CCTCTGGAGGAAGTGCTA-3′ [SEQ ID NO: 19]; human IL-3 exon 1/exon 3 junction-specific 5′-CCTTTGCTGGAAAATAACC-3′ [SEQ ID NO: 20]; human IL-5 exon 1/exon 3 junction-specific 5′-GCCAATGAGCACCAACTG-3′ [SEQ ID NO: 21]; and human GM-CSF exon 1/exon 3 junction-specific 5′-GCTGAGATGGAGCCGACC-3′ [SEQ ID NO: 22].

Two IL-4 mRNA species were consistently detected from all donors tested (Fig. 1). The larger IL-4 RT-PCR amplification product was 362 bp, corresponding to the predicted size of IL-4 mRNA. The second, smaller RT-PCR amplification product, designated IL-4δ2, migrated with an apparent size of 314 bp. Changes in the PCR buffer

sequences determined (Fig. 3). Sequence analysis of IL-4δ2 cDNA demonstrated the presence of IL-4 exons 1, 3 and 4, with exon 1 spliced directly to exon 3. Sequence analysis of IL-4 cDNA isolated, cloned, and sequenced in parallel with IL-4δ2 cDNA demonstrated the expected presence of exons 1, 2, 3 and 4, with a exon 2 to exon 3 in-frame splice junction. Of note, both IL-4 and IL-4δ2 contain gaa residues 5′ at exon 2-exon 3 and exon 1-exon 3 splices, respectively. No other sequence changes were observed throughout the entire protein-encoding region of IL-4δ2.

EXAMPLE 8

IL-4δ2 mRNA Expression in Healthy Humans and in Human T Cell Clones.

IL-4 and IL-4δ2 mRNA expression were analyzed in PBMC from 25 healthy humans. IL-4 and IL-4δ2 mRNA were co-expressed in all donors tested, but varied in relative ratio from individual to individual. Examples of this variability are shown in Fig. 5. In this experiment, PBMC from 3 individuals were stimulated with anti-CD3 MAb for 6 hours. The relative expression of IL-4 to IL-4δ2 mRNA was measured by RT-PCR using conditions under which the PCR products were being exponentially amplified (25 cycles). The ratio of IL-4:IL-4δ2 mRNA varied from approximately 2:1 in individual 1 to 1:2 in individual 3. Individual 2 expressed approximately equal amounts of IL-

had returned to baseline. At 24 and 48 hours, ratios of IL-4 to IL-4δ2 mRNA remained at the baseline of approximately 4 to 1 (data not shown).

EXAMPLE 10

Absence of IL-4δ2 mRNA in Mice.

The human and murine IL-4 genes are each composed of 4 exons and 3 introns, both with a 48 bp exon 2. To determine whether mice also express an alternatively spliced variant of IL-4 with exon 2 deleted, spleen cells from BALB/c mice were stimulated with PMA and ionomycin for 24 hours. RNA was extracted and subjected to RT-PCR using murine IL-4 exon 1- and exon 4-specific primers. Human IL-4δ2 mRNA expression was assayed in parallel from anti-CD3 MAb stimulated PBMC. IL-4, but not IL-4δ2, mRNA expression was observed in stimulated murine spleen cells, whereas human PBMC expressed both IL-4 and IL-4δ2 mRNA (Fig. 8).

EXAMPLE 11

Alternative Splicing of Exon 2 is Also Observed for Human IL-2 mRNA but not Human IL-3, IL-5 and GM-CSF mRNAs.

Because IL-4 belongs to a multigene family of cytokines, IL-2, IL-3, IL-5, and GM-CSF mRNAs were examined to determine whether alternative splicing is used to produce variants that are missing exon 2. Total RNA isolated from human PBMC stimulated for 6 hours with

hybridized with an IL-2 exon 1/exon 3 junctional probe (panel C). Because portions of the probe were homologous to exon 1 or exon 3, native IL-2 cDNA was detected with this probe as a larger 458 bp band on the autoradiogram. However, because this probe contained the exon 1/exon 3 junction, IL-2δ2 mRNA was easily discerned as a smaller 398 bp band.

In similar studies, the RT-PCR products for IL-3, IL-5 and GM-CSF were size separated by gel electrophoresis, transferred to a nylon membrane, and hybridized with oligonucleotide probes encoding an exon 1/exon 3 junctional sequence for IL-3, IL-5 and GM-CSF, respectively. No RT-PCR products hybridized with the IL-3, IL-5 or GM-CSF exon 1/exon 3 specific probes (data not shown).

EXAMPLE 12

Rabbit antisera specific for IL-4δ2 protein

A synthetic 16-mer peptide LNSLTEQKNTTEKETF (SEQ ID NO:27) was made. This peptide is specific for the exon 1-exon 3 junction in IL-4δ2 and is not present in IL-4. This peptide was made multimeric through coupling to MAPs resin. Purified multimeric peptide was used to immunize and boost two rabbits, a total of three injections. The post-immunization, but not preimmunization sera from each rabbit binds the IL-4δ2 synthetic peptide, but not recombinant human IL-4 or IL-2, in Western blots.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Alms, William et al

(ii) TITLE OF INVENTION: HUMAN INTERLEUKIN VARIANTS GENERATED BY ALTERNATIVE SPLICING

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Burns, Doane, Swecker & Mathis
(B) STREET: P.O. Box 1404
(C) CITY: Alexandria
(D) STATE: Virginia
(E) COUNTRY: United States
(F) ZIP: 22313-1404

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: To be assigned
(B) FILING DATE: Even date herewith
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Crane-Feury, Sharon E
(B) REGISTRATION NUMBER: 36,113

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (703) 836-6620
(B) TELEFAX: (703) 836-2021

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

ATGTACAGGA TGCAACTCCT GTCTT 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

CGTACTCTGG TTGGCTTCC 19

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

AAGCTTATGG GTCTCACCTC CCAAC 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GGATCCTCAT CAGCTCGAAC ACTTTGA 27

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AGCCATATCC ACGGATGCGA C 21

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCACTCCTGG ACTGGCTCCC AGCA 24

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CAGCTCTGCA TCGTTTTGGG TTCT 24

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGCTCTTCGA CCTTGAAACA GCAT 24

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTAAAACGAC GGCCAGT 17

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GCTGAGATGG AGCCGACC 18

10. The isolated nucleic acid of Claim 8, wherein the nucleic acid is DNA.

11. An expression vector comprising the isolated nucleic acid of Claim 10.

12. A transformed cell comprising the vector of Claim 11.

13. The polypeptide expressed by the expression vector of Claim 12.

14. An antibody directed to the polypeptide of Claim 13.

15. A method of regulating the activity of interleukin-4, comprising administering to a human an amount of the polypeptide of Claim 6 effective to decrease the biological effects of interleukin-4.

16. A method of regulating the activity of interleukin-2, comprising administering to a human an amount of the polypeptide of Claim 13 effective to decrease the biological effects of interleukin-2.

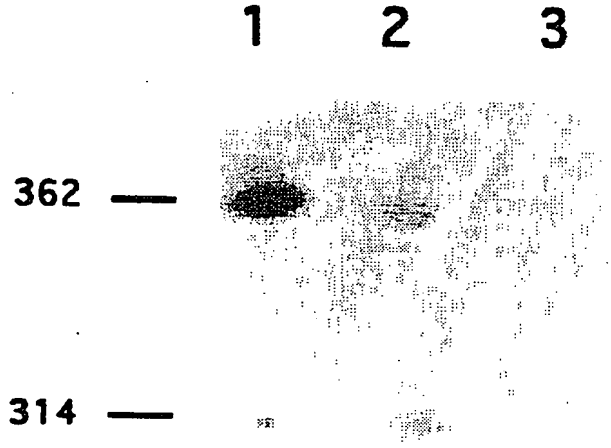

FIG. 2

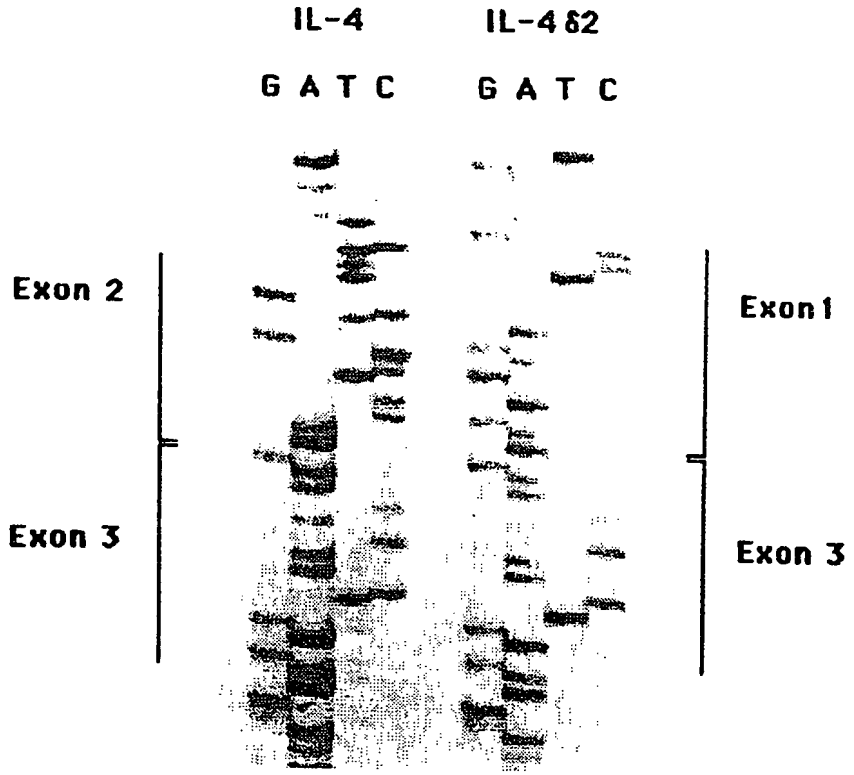

FIG. 3

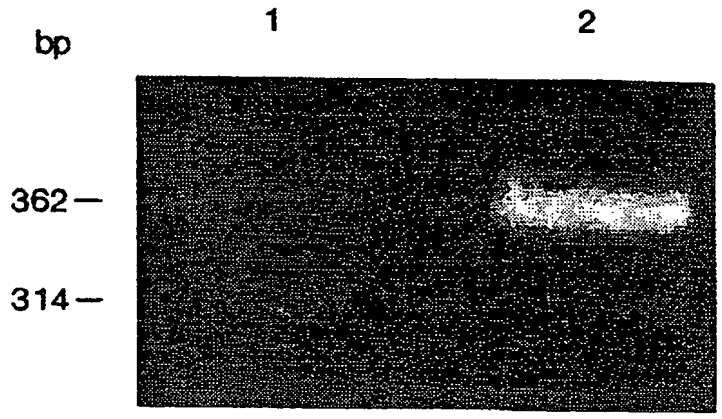

FIG. 6

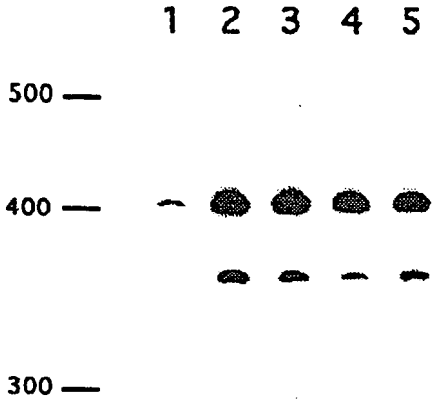

FIG. 7

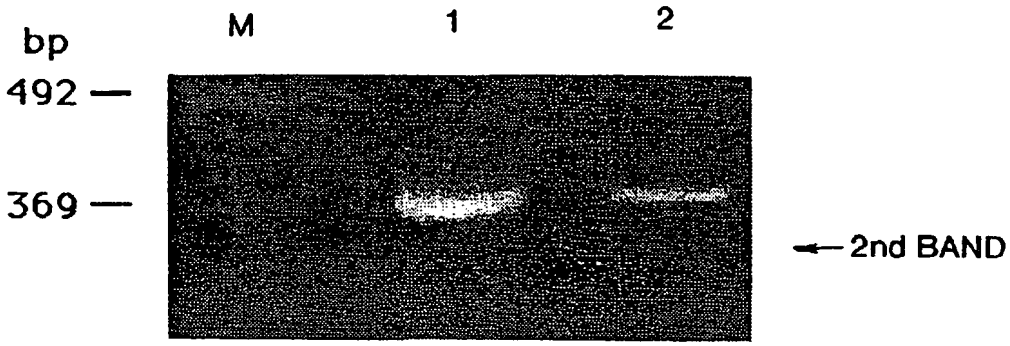

FIG. 8

gaattcaataaaaaacaagcagggcgcgtggtggggcactgactaggagggctgatttgtaagttggtaacactgtagctctttttcctaattagctgaggatgtgtttaggttccattc 120
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Exon 1 MATURE PROTEIN STARTS WITH H |W G L T S Q L L P P

ctgttcttcctgctagcatgtgccggcaactttgtccacggacacaagtgcgatatcaccttacaggagatcatcaaaactttgaacagcctcacagagcagaaggtgagtacctatctg 1320
L F F L L A C A G N F V M G|H K C D I T L Q C I I K T L N S L T E Q K|

gcaccatctctccagatgttctggtgatgctctcagtatttctaggcatgaaaacgttaacagctgctagagaagttggaactggtggttggtggcagtccagggcacacagagaggctt 1440
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Exon 2 |T L C T E L T V T D I F A A S K|
tctgaacaagaaacttgtctaatggaaaacgagcgggcccaaattaactctaaggtgttagatgtttcaaagaacgagaagtctgatctttactcataagcatgttttggtctttctgg 1800
tttcacttgatttagaagacatgtaatagaaagcttacatgctgtagtcctgactcagatcctggtcaaagaaaagccctcttgggttttacttagctttggcatagtgcctggaacgta 1920
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FIG. 10A

```
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                        Exon 1             MATURE PROTEIN STARTS WITH H                      |W  G  L  T  S  Q  L  L  P  P

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 L  F  F  L  L  A  C  A  G  N  F  V  M  G|H  K  C  D  I  T  L  Q  C  I  I  K  T  L  N  S  L  T  E  Q  K|

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    Exon 2      |T  L  C  T  E  L  T  V  T  D  I  F  A  A  S  K|
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FIG. 11A

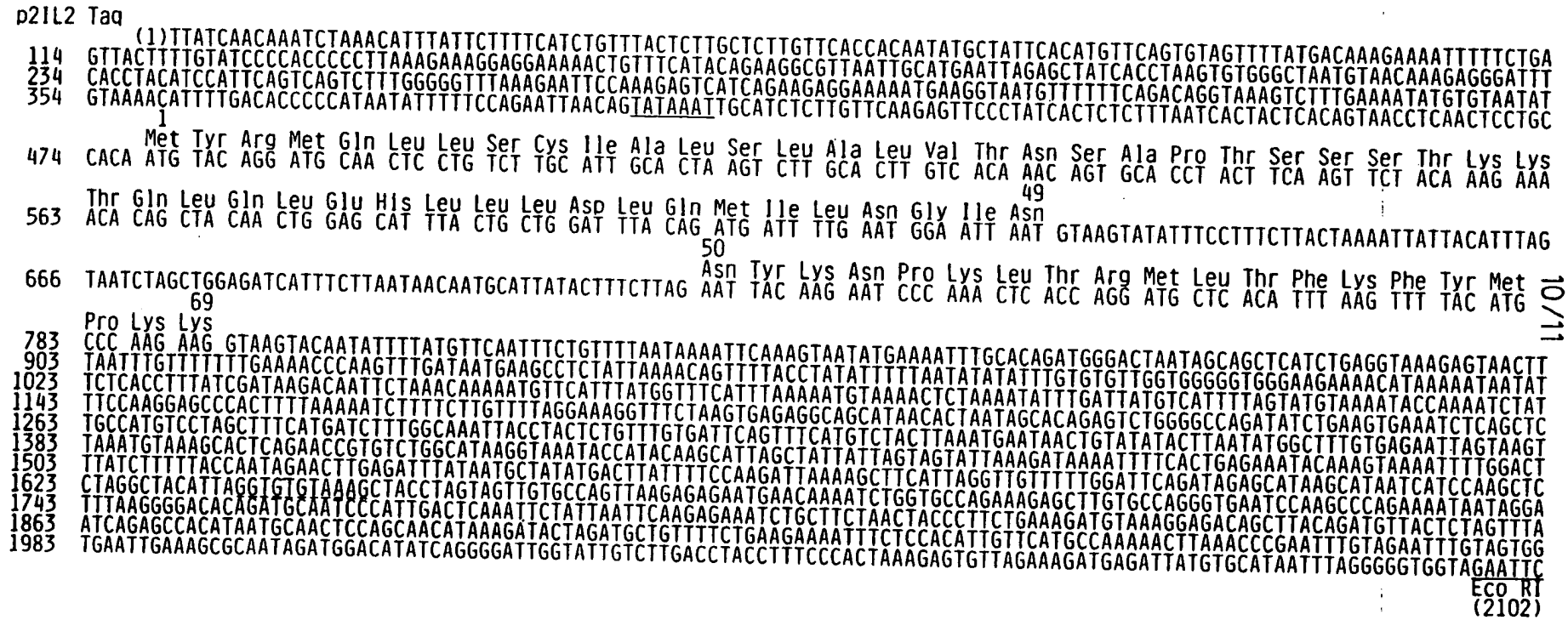

FIG. 12A

INTERNATIONAL SEARCH REPORT

I. ational application No.
PCT/US95/04094

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00, 1/20; A61K 38/20; C07K 14/52, 16/24

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.1; 530/387.1, 351; 424/85.2, 514/1; 435/69.52, 252.3; 930/141

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CAS ONLINE, MEDLINE, BIOSIS, SCISEARCH
search terms: interleukin-2, interleukin-4, alternative splicing, variant, antibody, treatment or administration.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EXPERIMENTAL HEMATOLOGY, Vol. 21, issued 1993, Sorg et al, "Identification of an Alternatively Spliced Transcript of Human Interleukin-4 Lacking the Sequence Encoded by Exon 2", pages 560-563, see page 560, paragraph 1.	1-3
X, P	IMMUNOGENETICS, Vol. 41, issued 1995, Klein et al, "An Alternatively Spliced Interleukin 4 Form in Lymphoid Cells", page 57, see entire document.	1-3

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 JUNE 1995	Date of mailing of the international search report 06 JUL 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PREMA MERTZ Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

I. ...national application No.
PCT/US95/04094

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.